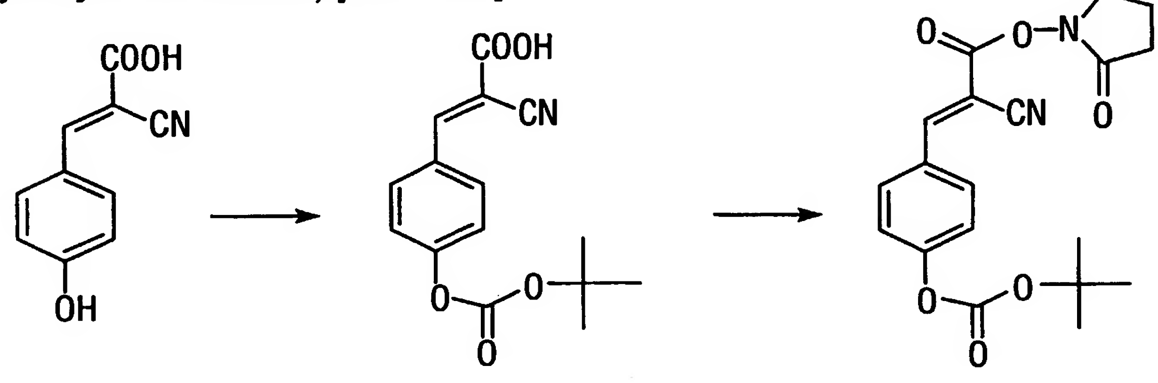
Fig.1.

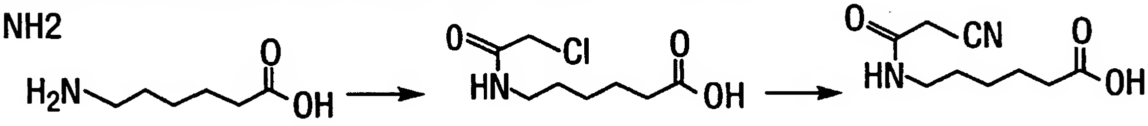
Synthesis of 2-Cyan-3-(4-tert-butoxycarbonyloxyphenyl) acrylic acid-N-(hydroxysuccinimidester) [NHS-HCCA]:



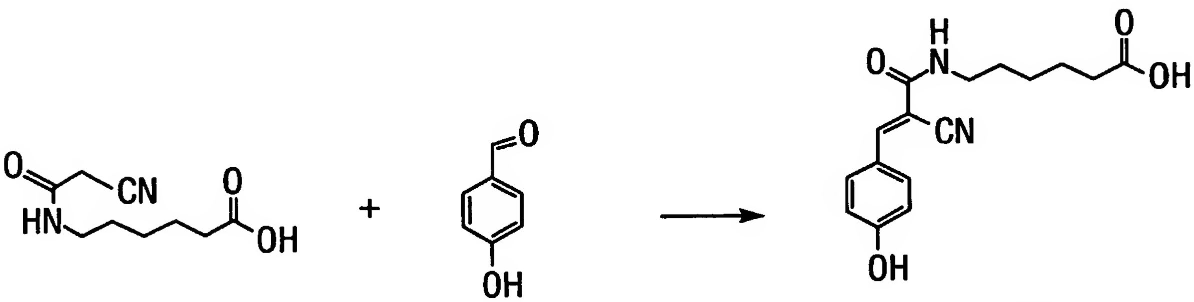
189,2 1 | 289,3 2 | 386,4 3

Synthesis of 2-Cyan-3-(4-tert-hydroxyphenyl) acrylic acid-6-[(2,5-dioxo-1-pyrrolidinyl) oxy)-(6-oxo) hexyl] amide [NHS-L-HCCA]:

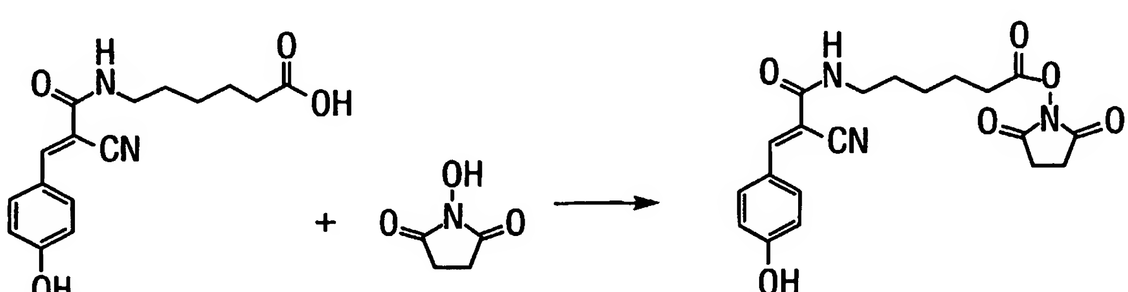
NH2



131 | 207.7 1 | 198.3 2



198.3 2 | 122.1 | 302.3 3



3 | 115.1 | 399.4 4

Fig.2.

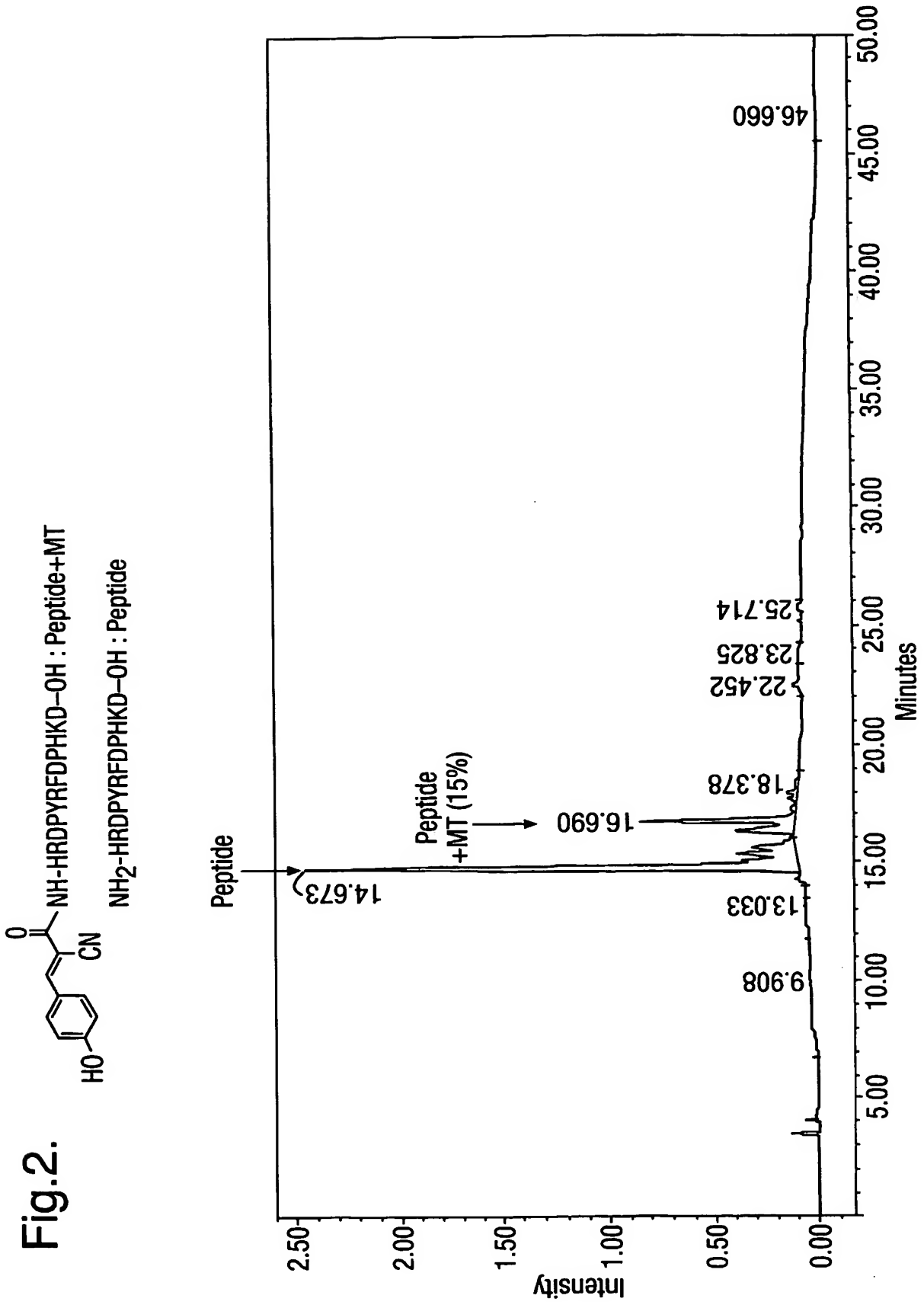

NH-HRDPYRFDPHKD–OH : Peptide+MT
NH2-HRDPYRFDPHKD–OH : Peptide
O
CN
HO
Peptide
Peptide +MT (15%)
14.673
16.690
18.378
22.452
23.825
25.714
46.660
9.908
13.033
Intensity
Minutes
0.00
0.50
1.00
1.50
2.00
2.50
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00

Fig.3.

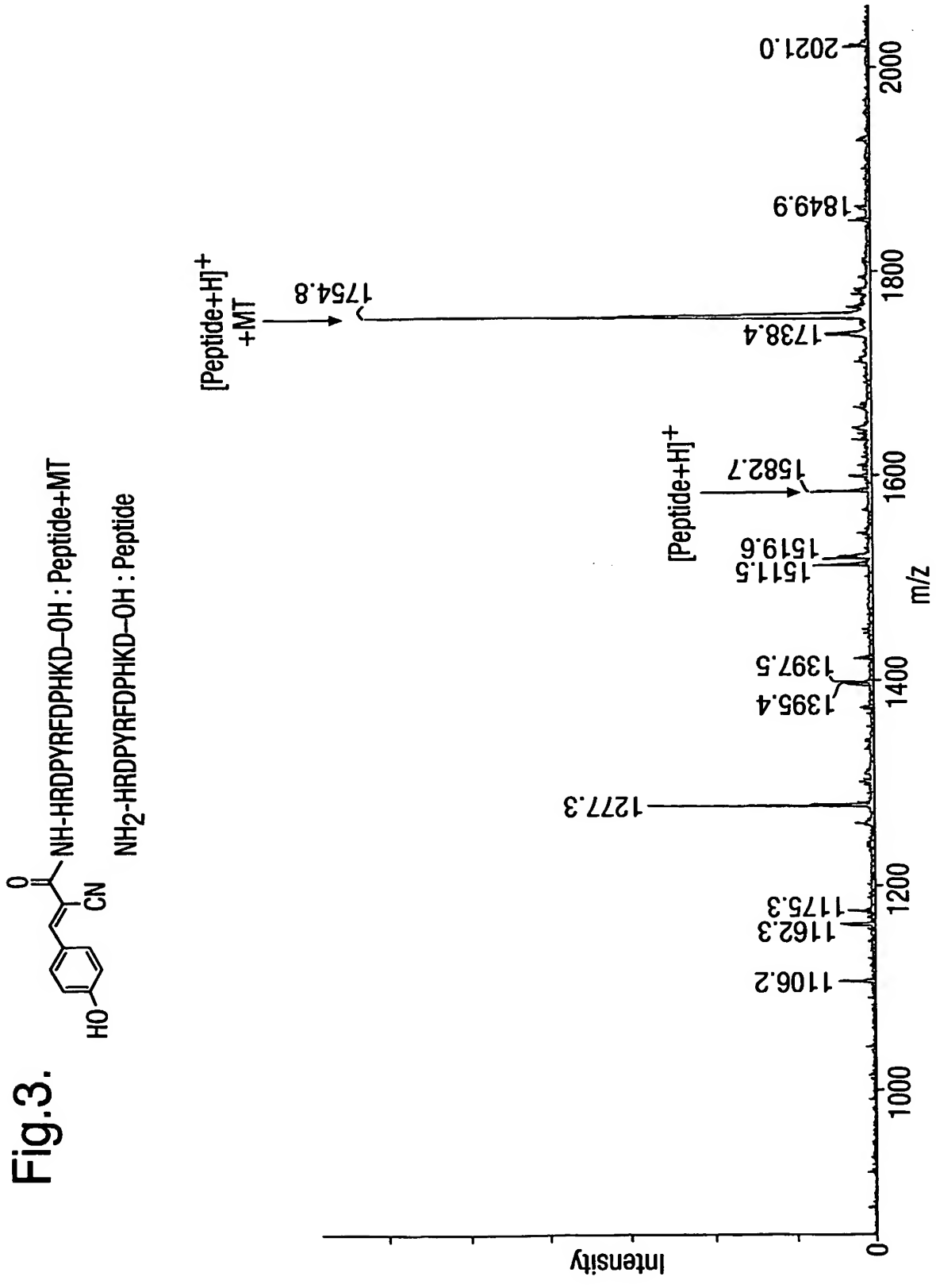

NH-HRDPYRFDPHKD–OH : Peptide+MT
NH2-HRDPYRFDPHKD–OH : Peptide
O
CN
HO
Intensity
m/z
0
1000
1200
1400
1600
1800
2000
2021.0
1849.9
1754.8
[Peptide+H]+ +MT
1738.4
1582.7
[Peptide+H]+
1519.6
1511.5
1397.5
1395.4
1277.3
1175.3
1162.3
1106.2

Fig.4.

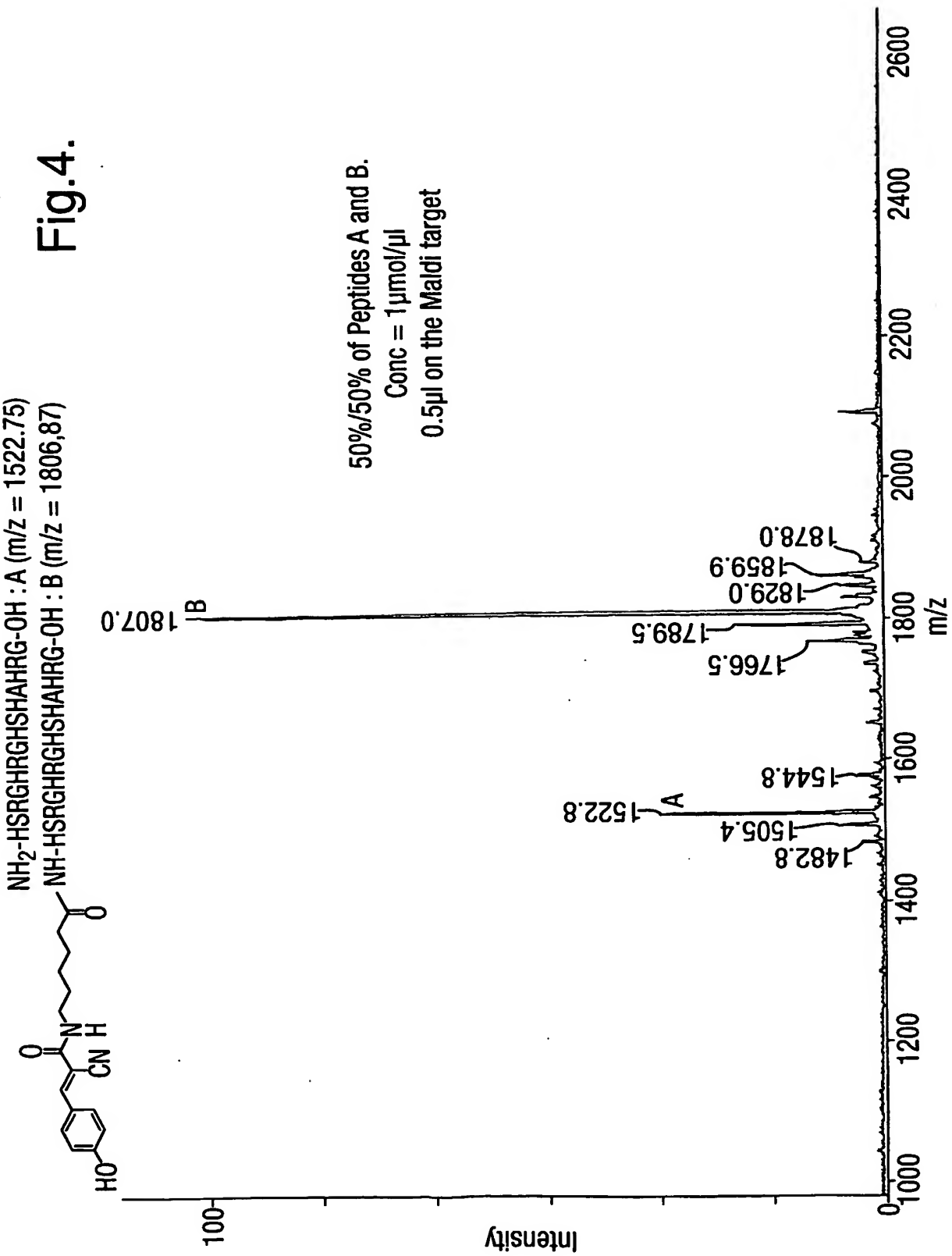

NH2-HSRGHRGHSHAHRG-OH : A (m/z = 1522.75)
NH-HSRGHRGHSHAHRG-OH : B (m/z = 1806,87)
50%/50% of Peptides A and B.
Conc = 1µmol/µl
0.5µl on the Maldi target
Intensity
m/z
100
0
1000
1200
1400
1600
1800
2000
2200
2400
2600
1482.8
1505.4
1522.8
A
1544.8
1766.5
1789.5
1807.0
B
1829.0
1859.9
1878.0
HO
CN H
N
H
O
O

Fig.5a.


1 6 11 18
NH2-DEGPYKMEHFRWGSPPKD-CO2H
1 6
NH2DEGPYK-CO2H
7 11
NH2-MEHFR-CO2H
12 18
NH2-WGSPPKD-CO2H
Matrix MT labelling
SMT
1 6
SMT–NH2-DEGPYK-CO2H
7 11
SMT–NH2-MEHFR-CO2H
12 18
SMT–NH2-WGSPPKD-CO2H
SMT

Fig.5b.

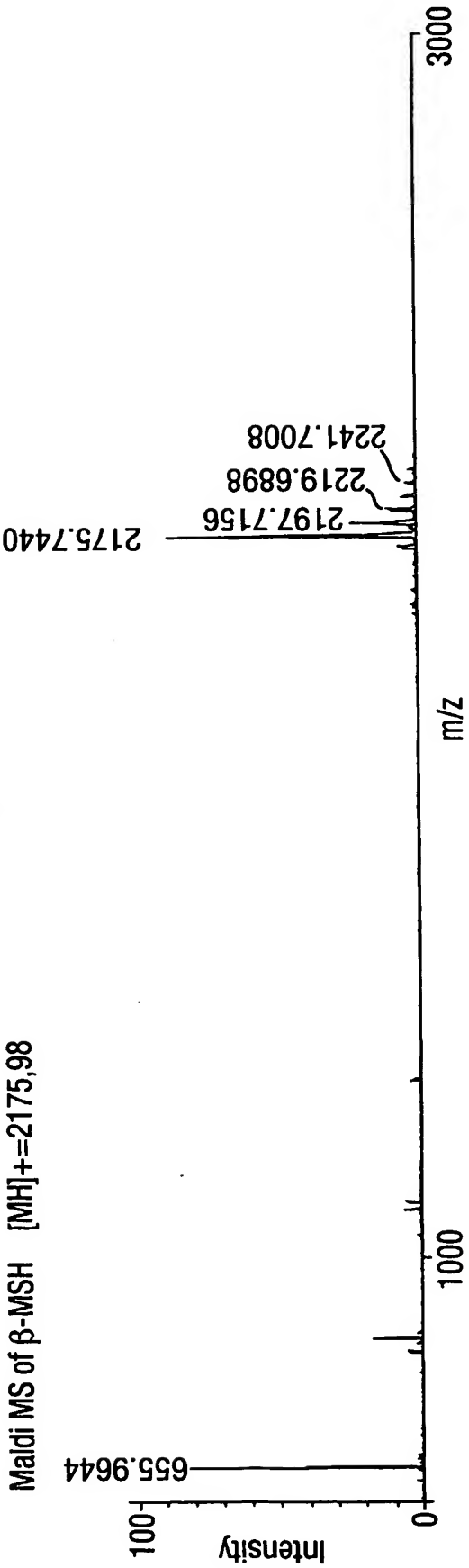

Maldi MS of β-MSH [MH]+=2175,98
655.9644
2175.7440
2197.7156
2219.6898
2241.7008
Intensity
100
0
1000
3000
m/z

Fig.6.

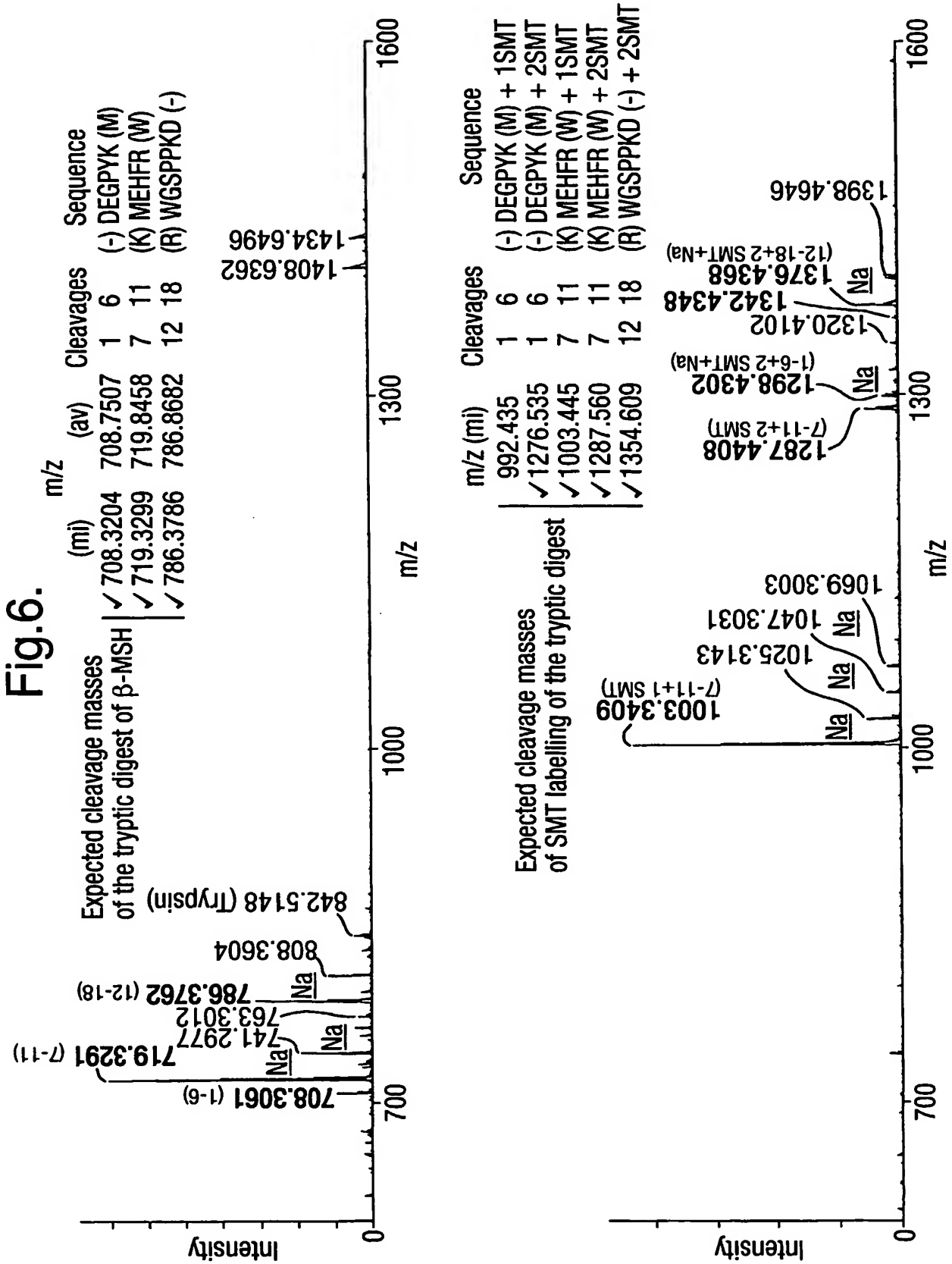

Expected cleavage masses of the tryptic digest of β-MSH
m/z (mi) m/z (av) Cleavages Sequence
✓ 708.3204 708.7507 1 6 (-) DEGPYK (M)
✓ 719.3299 719.8458 7 11 (K) MEHFR (W)
✓ 786.3786 786.8682 12 18 (R) WGSPPKD (-)
842.5148 (Trypsin)
808.3604
786.3762 (12-18)
763.3012
741.2977
719.3291 (7-11)
708.3061 (1-6)
1434.6496
1408.6362
Intensity
m/z
0
700
1000
1300
1600
Expected cleavage masses of SMT labelling of the tryptic digest
m/z (mi) Cleavages Sequence
992.435 1 6 (-) DEGPYK (M) + 1SMT
✓1276.535 1 6 (-) DEGPYK (M) + 2SMT
✓1003.445 7 11 (K) MEHFR (W) + 1SMT
✓1287.560 7 11 (K) MEHFR (W) + 2SMT
✓1354.609 12 18 (R) WGSPPKD (-) + 2SMT
1398.4646
1376.4368 (12-18+2 SMT+Na)
1342.4348
1320.4102
1298.4302 (1-6+2 SMT+Na)
1287.4408 (7-11+2 SMT)
1069.3003
1047.3031
1025.3143
1003.3409 (7-11+1 SMT)
Intensity
m/z
0
700
1000
1300
1600

Fig.7.

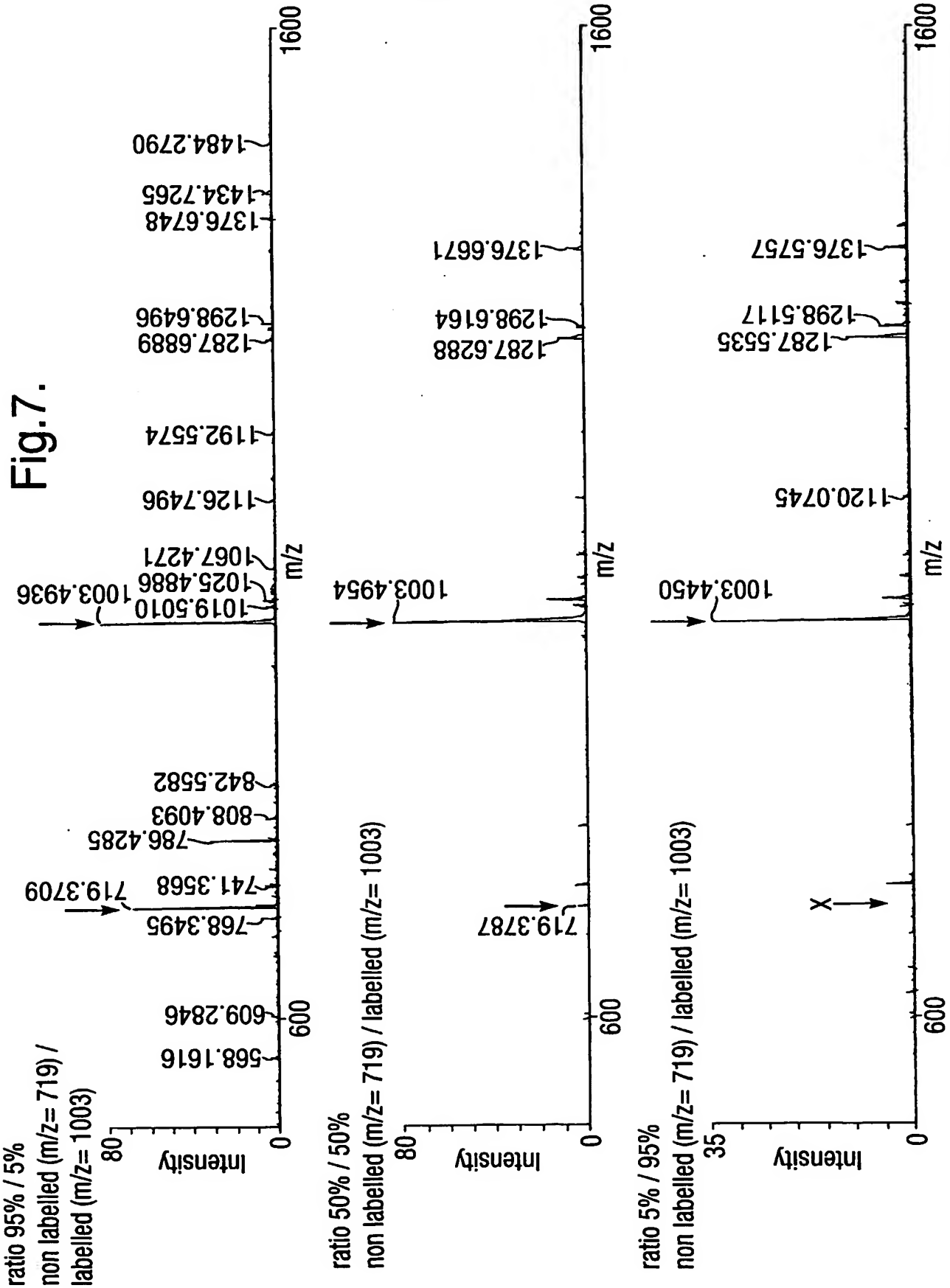

ratio 95% / 5%
non labelled (m/z= 719) /
labelled (m/z= 1003)
Intensity
m/z
568.1616
609.2846
768.3495
719.3709
741.3568
786.4285
808.4093
842.5582
1003.4936
1019.5010
1025.4886
1067.4271
1126.7496
1192.5574
1287.6889
1298.6496
1376.6748
1434.7265
1484.2790
ratio 50% / 50%
non labelled (m/z= 719) / labelled (m/z= 1003)
719.3787
1003.4954
1287.6288
1298.6164
1376.6671
ratio 5% / 95%
non labelled (m/z= 719) / labelled (m/z= 1003)
X
1003.4450
1120.0745
1287.5535
1298.5117
1376.5757

Fig.8.

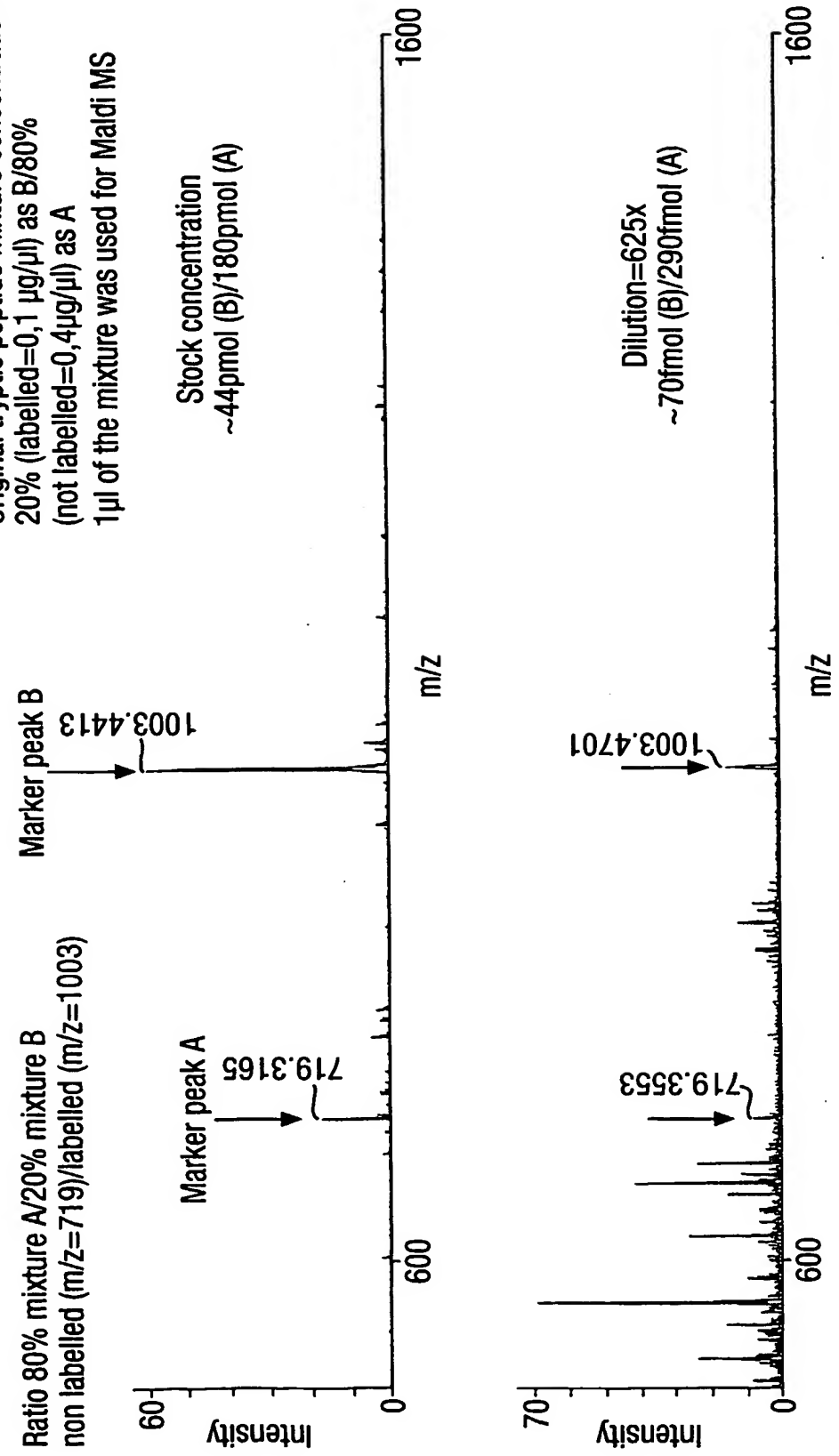

Original tryptic peptide mixture concentration:
20% (labelled=0,1 µg/µl) as B/80%
(not labelled=0,4µg/µl) as A
1µl of the mixture was used for Maldi MS
Ratio 80% mixture A/20% mixture B
non labelled (m/z=719)/labelled (m/z=1003)
Marker peak B
1003.4413
Marker peak A
719.3165
Stock concentration
~44pmol (B)/180pmol (A)
Intensity
60
0
600
1600
m/z
Dilution=625x
~70fmol (B)/290fmol (A)
1003.4701
719.3553
Intensity
70
0
600
1600
m/z

Fig.8 (Cont).

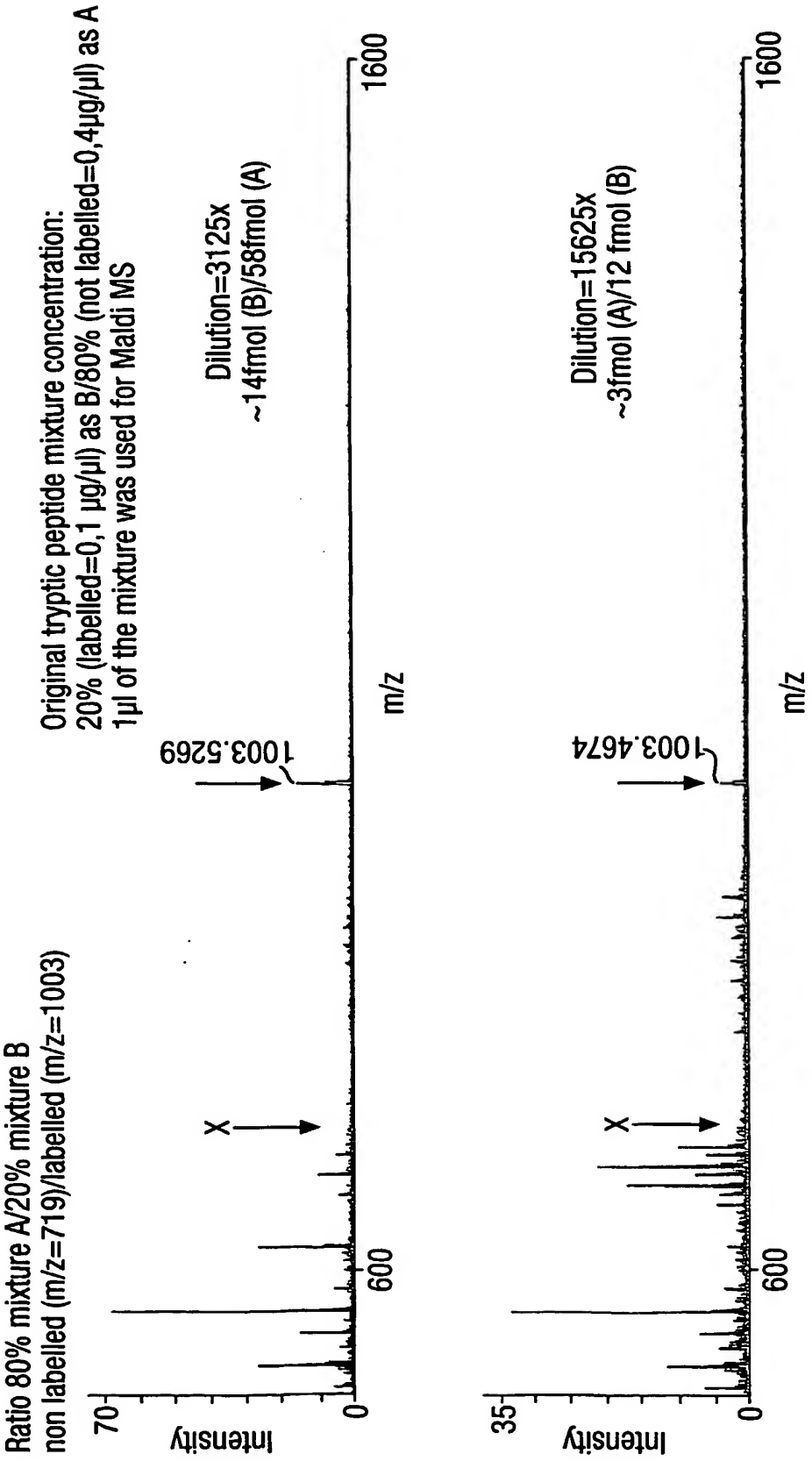

Ratio 80% mixture A/20% mixture B
non labelled (m/z=719)/labelled (m/z=1003)
Original tryptic peptide mixture concentration:
20% (labelled=0,1 µg/µl) as B/80% (not labelled=0,4µg/µl) as A
1µl of the mixture was used for Maldi MS
Dilution=3125x
~14fmol (B)/58fmol (A)
1003.5269
X
Intensity
70
0
600
1600
m/z
Dilution=15625x
~3fmol (A)/12 fmol (B)
1003.4674
X
Intensity
35
0
600
1600
m/z

Fig.9.

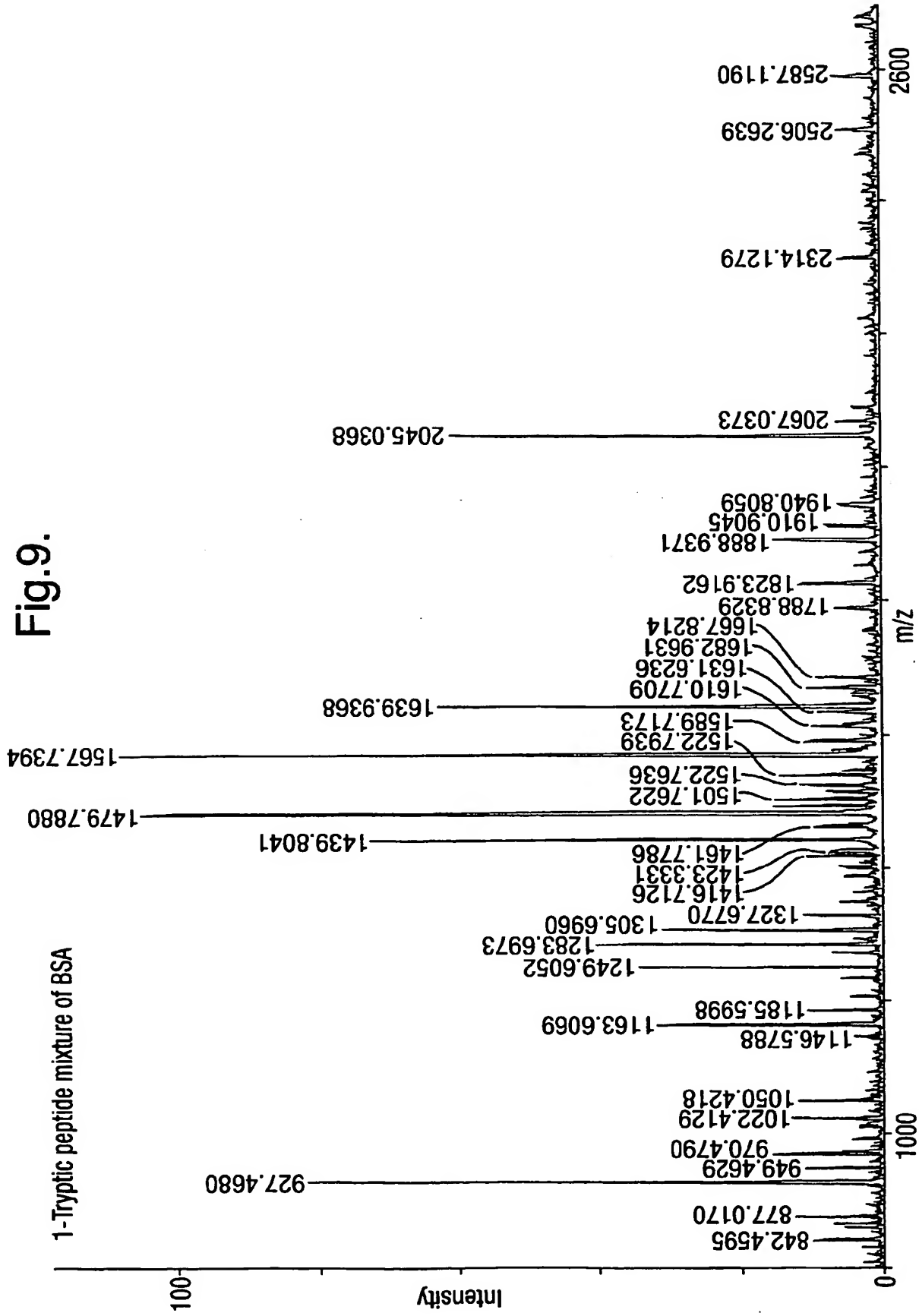

1-Tryptic peptide mixture of BSA
Intensity
100
0
m/z
1000
2600
842.4595
877.0170
927.4680
949.4629
970.4790
1022.4129
1050.4218
1146.5788
1163.6069
1185.5998
1249.6052
1283.6973
1305.6960
1327.6770
1416.7126
1423.3331
1461.7786
1439.8041
1479.7880
1501.7622
1522.7636
1522.7939
1567.7394
1589.7173
1610.7709
1631.6236
1639.9368
1682.9631
1667.8214
1788.8329
1823.9162
1888.9371
1910.9045
1940.8059
2045.0368
2067.0373
2314.1279
2506.2639
2587.1190

Fig.10.

15/42 matches (35%). 69293.9 Da, pI=5.82. Acc. # swiss:P02769. BOVIN. SERUM ALBUMIN PRECURSOR..

m/z submitted	MH^+ matched	Δppm	start	end	Peptide Sequence	Modifications
927.4680	927.4940	-28.0249	161	167	(K)YLYEIAR (R)	
1163.6069	1163.6312	-20.8956	66	75	(K)LVNELTEFAK (T)	
1249.6052	1249.6217	-13.2142	35	44	(R)FKDLGEEHFK (G)	
1283.6973	1283.7112	-10.8252	361	371	(R)HPEYAVSVLLR (L)	
1305.6960	1305.7167	-15.8348	402	412	(K)HLVDEPQNLIK (Q)	
1439.8041	1439.8123	-5.7004	360	371	(R)RHPEYAVSVLLR (L)	
1479.7880	1479.7960	-5.3985	421	433	(K)LGEYGFQNALIVR (Y)	
1567.7394	1567.7433	-2.4778	347	359	(K)DAFLGSFLYEYSR (R)	
1639.9368	1639.9383	-0.9122	437	451	(R)KVPQVSTPTLVEVSR (S)	
1667.8214	1667.8137	4.6234	469	482	(R)MPCTEDYLSLILNR (L)	
1823.9162	1823.9002	8.7746	508	523	(R)RPCFSALTPDETYVPK (A)	
1888.9371	1888.9274	5.1368	169	183	(R)HPYFYAPELLYYANK (Y)	
2045.0368	2045.0285	4.0547	168	183	(R)RHPYFYAPELLYYANK (Y)	
2506.2639	2506.2508	5.2276	469	489	(R)MPCTEDYLSLILNRLCVLHEK (T)	1Met-ox
3395.6670	3395.6438	6.8340	558	587	(K)HKPKATEEQLKTVMENFVAFVDKCCAADDK (E)	

The matched peptides cover **28%** (174/607 AA's) of the protein

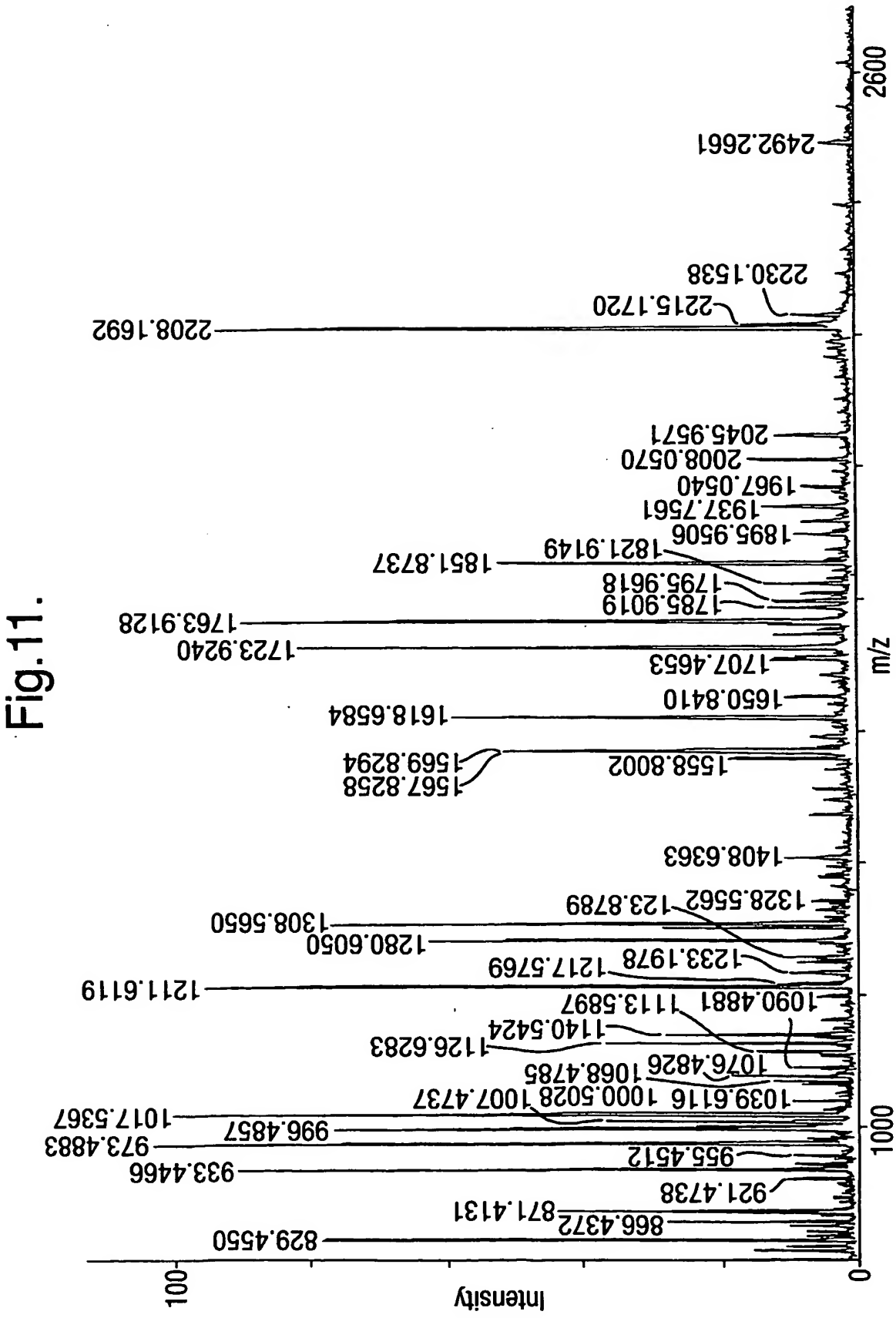

Intensity
100
0
m/z
1000
2600
829.4550
866.4372
871.4131
921.4738
933.4466
955.4512
973.4883
996.4857
1017.5367
1039.6116
1000.5028
1007.4737
1076.4826
1068.4785
1126.6283
1140.5424
1090.4881
1113.5897
1211.6119
1233.1978
1217.5769
1280.6050
1308.5650
1328.5562
123.8789
1408.6363
1558.8002
1567.8258
1569.8294
1618.6584
1650.8410
1707.4653
1723.9240
1763.9128
1785.9019
1795.9618
1821.9149
1851.8737
1895.9506
1937.7561
1967.0540
2008.0570
2045.9571
2208.1692
2215.1720
2230.1538
2492.2661

Fig.11.

Fig.12.

16/50 matches (32%). 69293.9 Da, pI = 5.82. Acc. # swiss: P02769. BOVIN. SERUM ALBUMIN PRECURSOR.

m/z submitted	MH^+ matched	Δ ppm	start	end	Peptide Sequence	Modifications
829.4550	829.4572	-2.6686	101	105	(K) VASLR (E)	1SMT
933.4466	933.4504	-4.0854	205	209	(K) IETMR (E)	1SMT
933.4466	933.4504	-4.0854	223	228	(R) CASIQK (F)	1SMT
973.4883	973.4896	-1.3110	236	241	(K) AWSVAR (L)	1SMT
996.4857	996.4903	-4.6207	29	34	(K) SEIAHR (F)	1SMT
1068.4785	1068.4791	-0.5345	25	28	(R) DTHK (S)	1SMT
1211.6119	1211.6101	1.4977	161	167	(K) YLYEIAR (R)	1SMT
1217.5769	1217.5665	8.5367	223	228	(R) CASIQK (F)	2SMT
1233.5978	1233.6097	-9.6395	156	160	(K)KFWGK (Y)	2SMT
1308.5650	1308.5717	-5.0955	499	507	(K) CCTESLVNR (R)	1SMT
1567.8258	1567.8273	-0.9497	361	371	(R) HPEYAVSVLLR (L)	1SMT
1569.8294	1569.8218	4.8352	233	241	(R) ALKAWSVAR (L)	2SMT
1723.9240	1723.9284	-2.5523	360	371	(R) RHPEYAVSVLLR (L)	1SMT
1763.9128	1763.9121	0.4075	421	433	(K) LGEYGFQNALIVR (Y)	1SMT
1795.9618	1795.9594	1.3222	438	451	(K) VPQVSTPTLVEVSR (S)	1SMT
1851.8737	1851.8594	7.7344	347	359	(K) DAFLGSFLYEYSR (R)	1SMT
2208.1692	2208.1705	-0.5801	437	451	(R) KVPQVSTPTLVEVSR (S)	2SMT

The matched peptides cover 17% (109/607 AA's) of the protein

Fig.13.

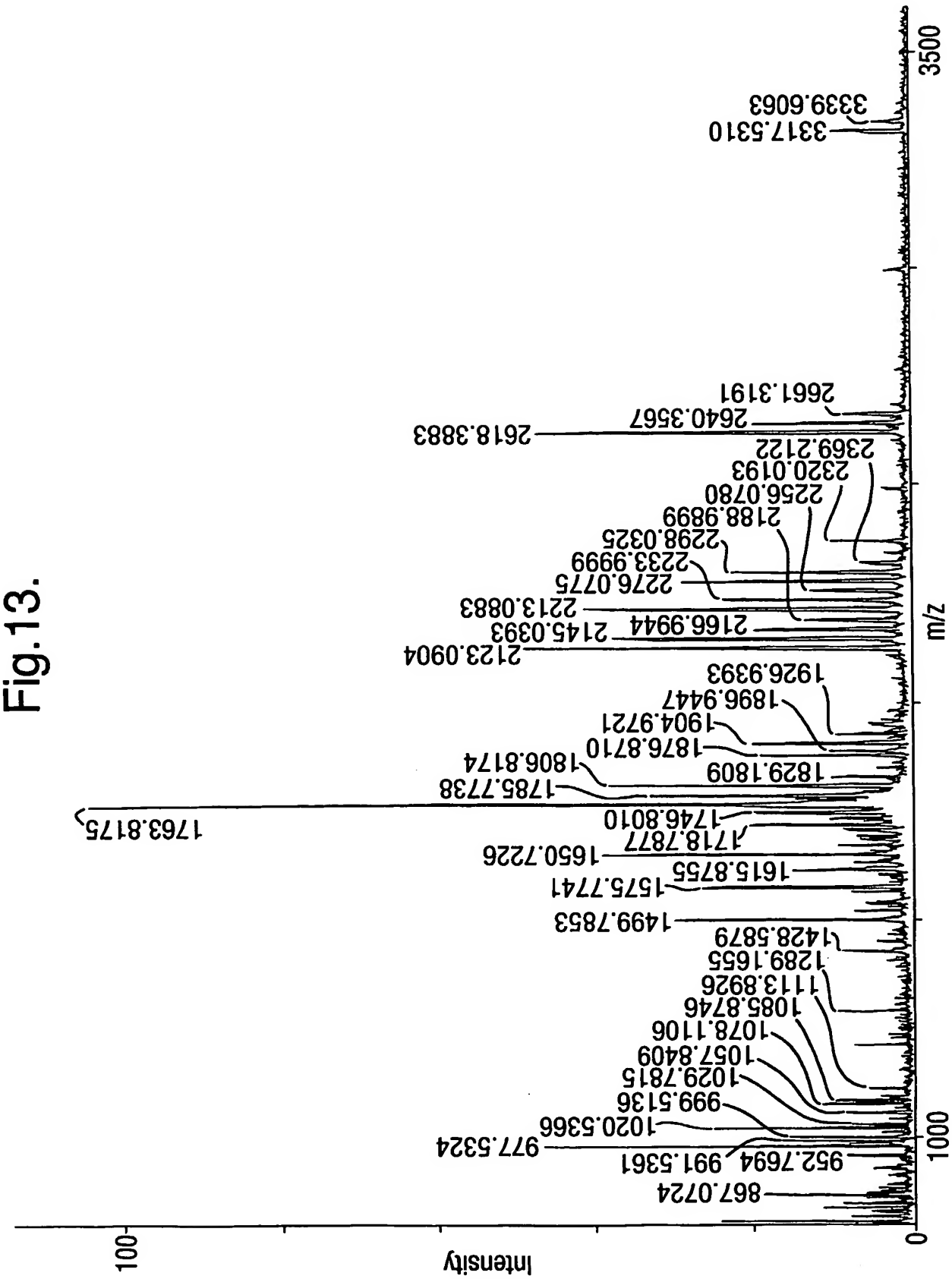

Intensity
100
0
m/z
1000
3500
867.0724
952.7694
991.5361
977.5324
1020.5366
999.5136
1029.7815
1057.8409
1078.1106
1085.8746
1113.8926
1289.1655
1428.5879
1499.7853
1575.7741
1615.8755
1650.7226
1718.7877
1746.8010
1763.8175
1785.7738
1806.8174
1829.1809
1876.8710
1904.9721
1896.9447
1926.9393
2123.0904
2145.0393
2166.9944
2213.0883
2276.0775
2233.9999
2298.0325
2188.9899
2256.0780
2320.0193
2369.2122
2618.3883
2640.3567
2661.3191
3317.5310
3339.6063

Fig.14.

7/56 matches (12%). 35691.1 Da, pI=8.52. Acc. # swiss:P46406. RABIT. GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE (EC 1.2.1.12) (GAPDH)..

m/z submitted	MH^+ matched	Δppm	start	end	Peptide Sequence	Modifications
977.5324	977.5420	-9.8265	70	77	(K)AITIFQER (D)	
1499.7853	1499.7892	-2.5929	232	245	(R)VPTPNVSVVDLTCR (L)	
1615.8755	1615.8808	-3.2682	70	83	(K)AITIFQERDPANIK (W)	
1763.8175	1763.8029	8.2636	307	320	(K)LISWYDNEFGYSNR (V)	
2618.3883	2618.3765	4.5189	160	183	(K)VIHDHFGIVEGLMTTVHAITATQK (T)	
3317.5310	3317.5651	-10.2886	25	52	(K)VDVVAINDPFIDLHYMVYMFQYDSTHGK (F)	
3339.6063	3339.6432	-11.0492	84	115	(K)WGDAGAEYVVESTGVFTTMEKAGAHLKGGAKR (V)	1Met-ox

The matched peptides cover **37%** (126/332 AA's) of the protein

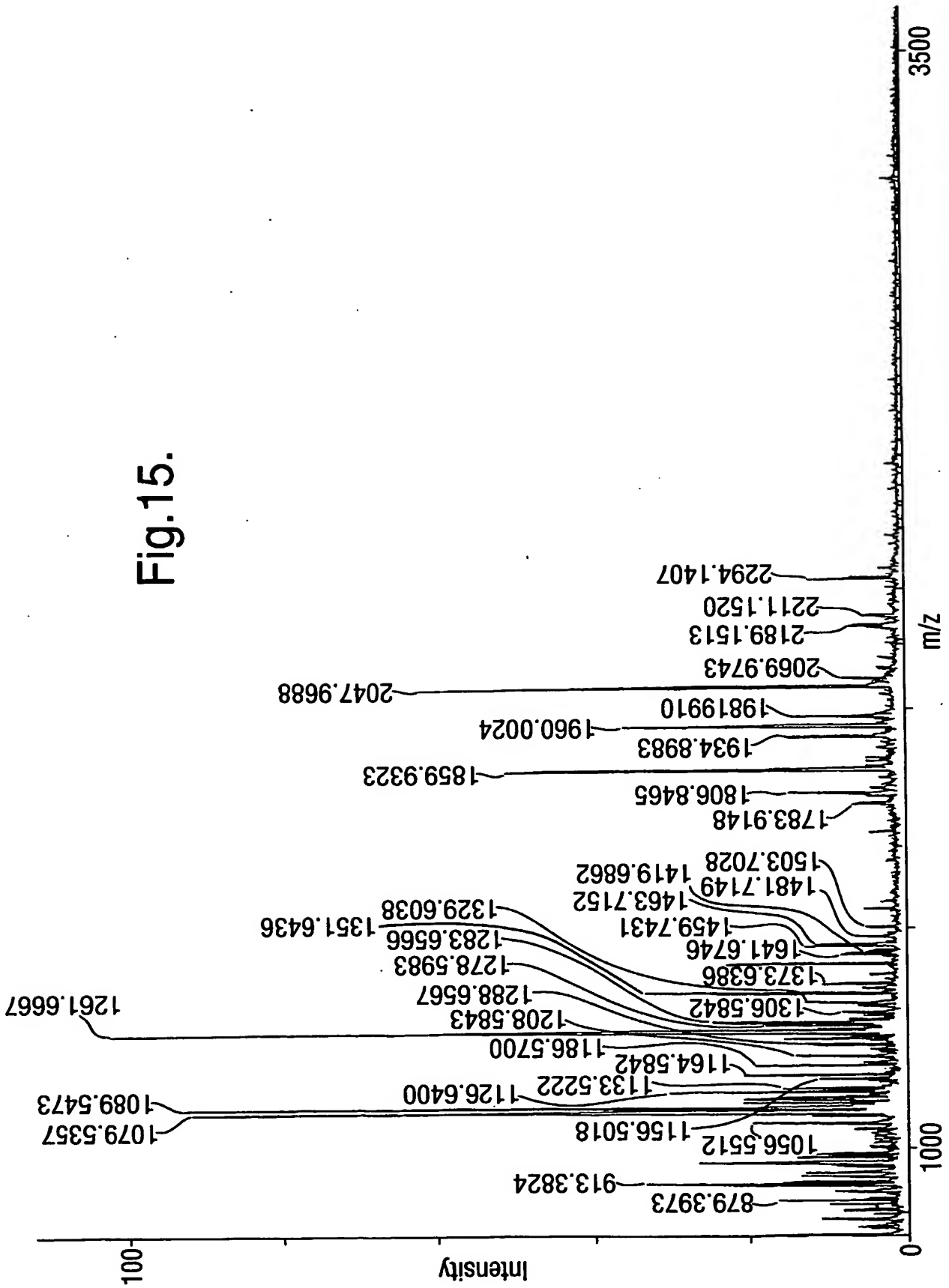

Intensity
100
0
m/z
3500
1000
879.3973
913.3824
1056.5512
1079.5357
1089.5473
1126.6400
1133.5222
1156.5018
1164.5842
1186.5700
1208.5843
1261.6667
1288.6567
1306.5842
1373.6386
1278.5983
1283.6566
1329.6038
1351.6436
1459.7431
1463.7152
1481.7149
1419.6862
1503.7028
1641.6746
1783.9148
1806.8465
1859.9323
1934.8983
1960.0024
2047.9688
1981991O
2069.9743
2189.1513
2211.1520
2294.1407

Fig.15.

Fig.16.

9/52 matches (17%). 35691.1 Da, pI=8.52. Acc. # swiss:P46406. RABIT. GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE (EC 1.2.1.12) (GAPDH)..

m/z submitted	MH^+ matched	Δppm	start	end	Peptide Sequence	Modifications
1056.5512	1056.5267	23.1986	111	115	(K)GGAKR(V)	2SMT
1079.5357	1079.5348	0.8227	225	231	(K)LTGMAFR (V)	1SMT
1089.5473	1089.5482	-0.7836	3	10	(K)VGVNGFGR (I)	1SMT
1164.5842	1164.5842	-0.0042	105	110	(K)AGAHLK(G)	2SMT
1261.6667	1261.6581	6.8178	70	77	(K)AITIFQER (D)	1SMT
1783.9148	1783.8808	19.0702	184	194	(K)TVDGPSGKLWR(D)	2SMT
1783.9148	1783.9053	5.3359	232	245	(R)VPTPNVSVVDLTCR (L)	1SMT
1960.0024	1959.9897	6.4986	252	260	(K)YDDIKKVVK(Q)	3SMT
1981.9910	1982.0096	-9.3660	195	212	(R)DGRGAAQNIIPASTGAAK (A)	1SMT
2047.9688	2047.9190	24.3090	307	320	(K)LISWYDNEFGYSNR (V)	1SMT

The matched peptides cover **30%** (100/332 AA's) of the protein

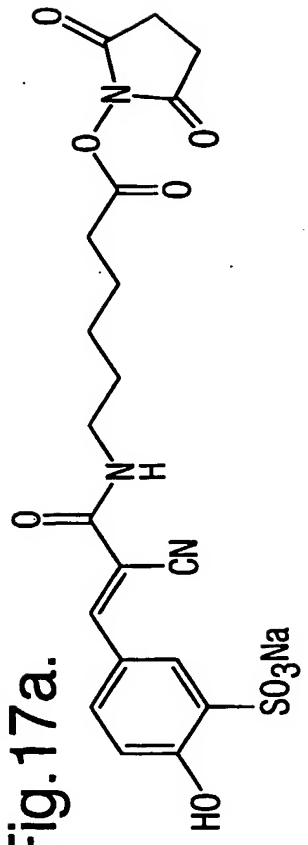


Fig.17a.

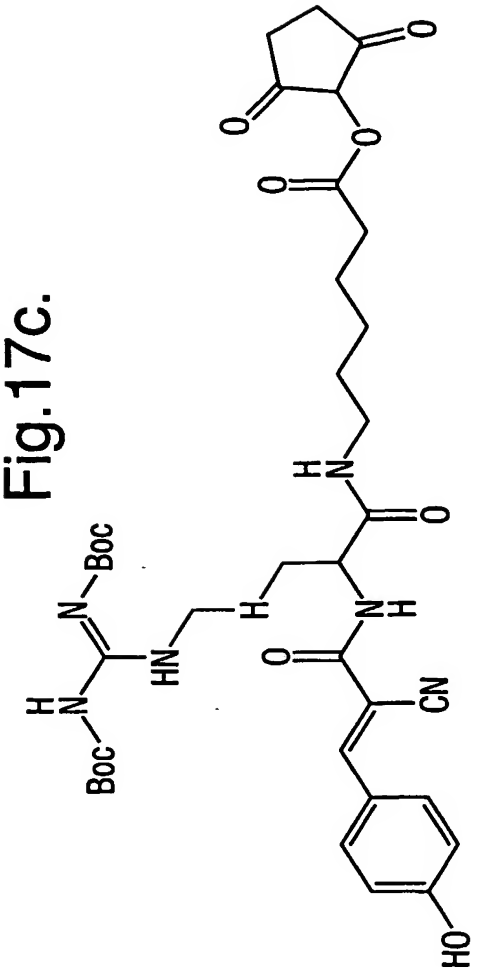


Fig.17c.

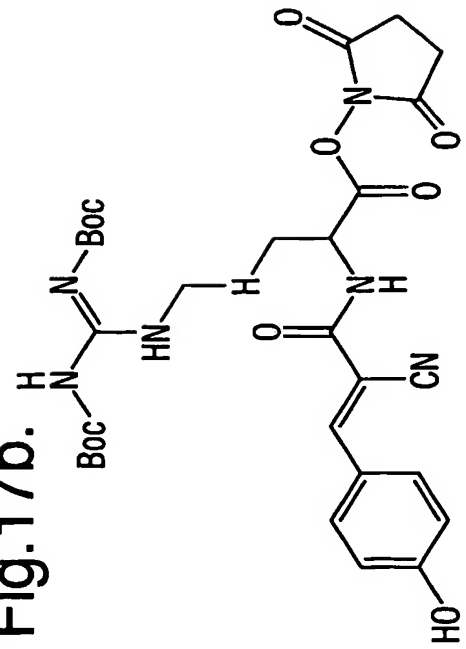


Fig.17b.

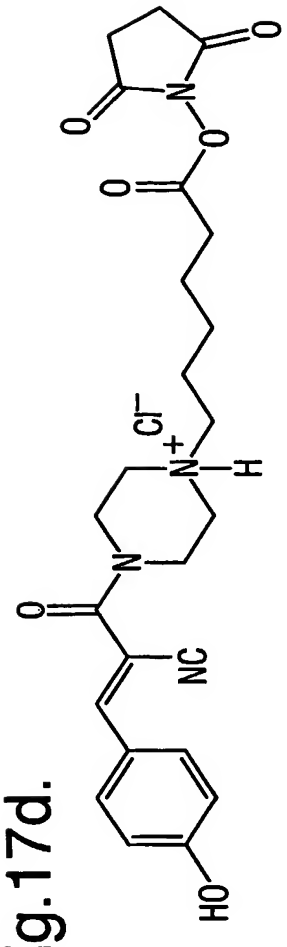
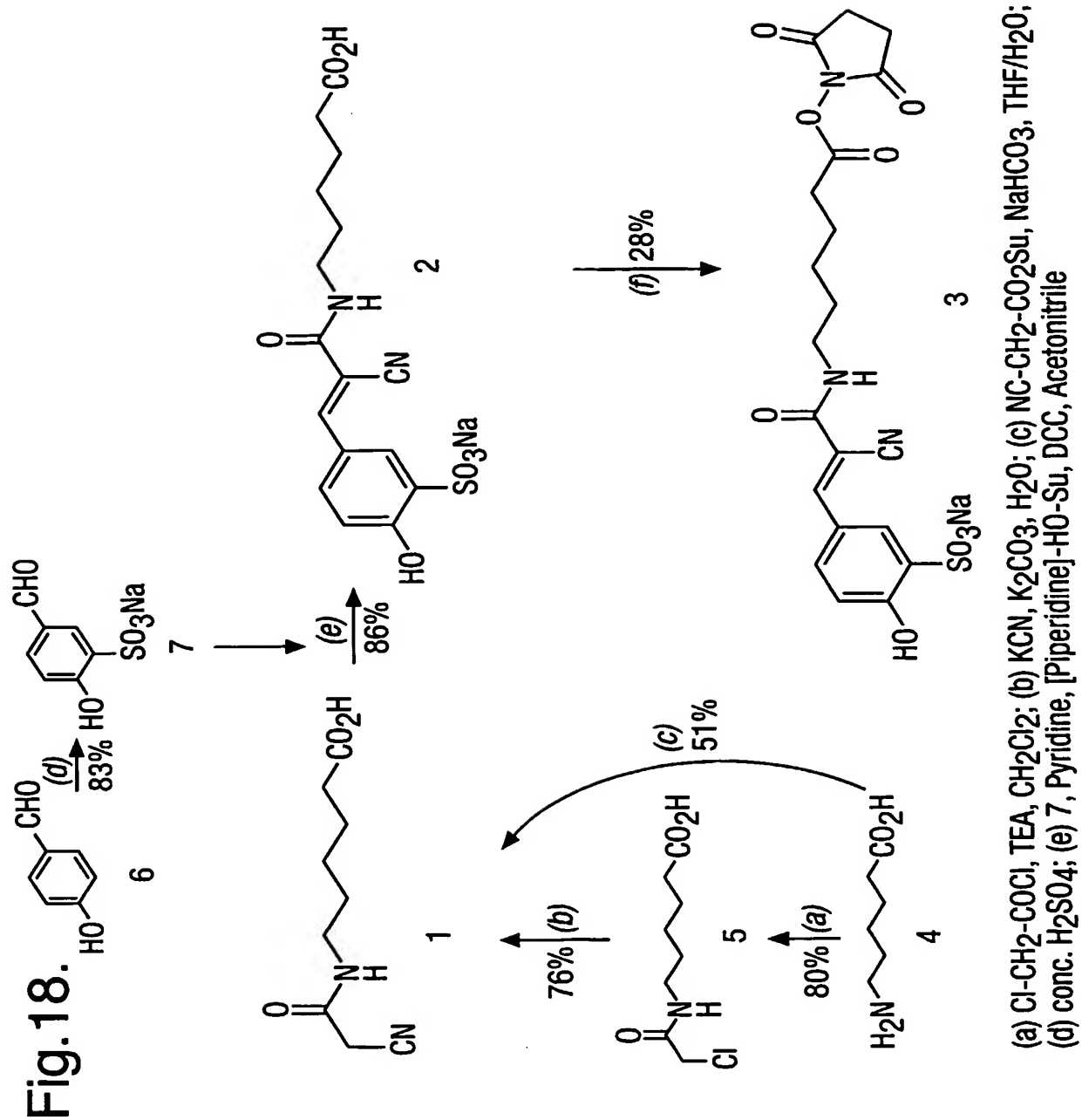


Fig.17d.

Fig.18.



(a) Cl-CH_2-COCl, TEA, CH_2Cl_2; (b) KCN, K_2CO_3, H_2O; (c) NC-CH_2-CO_2Su, $NaHCO_3$, THF/H_2O;
(d) conc. H_2SO_4; (e) 7, Pyridine, [Piperidine]-HO-Su, DCC, Acetonitrile

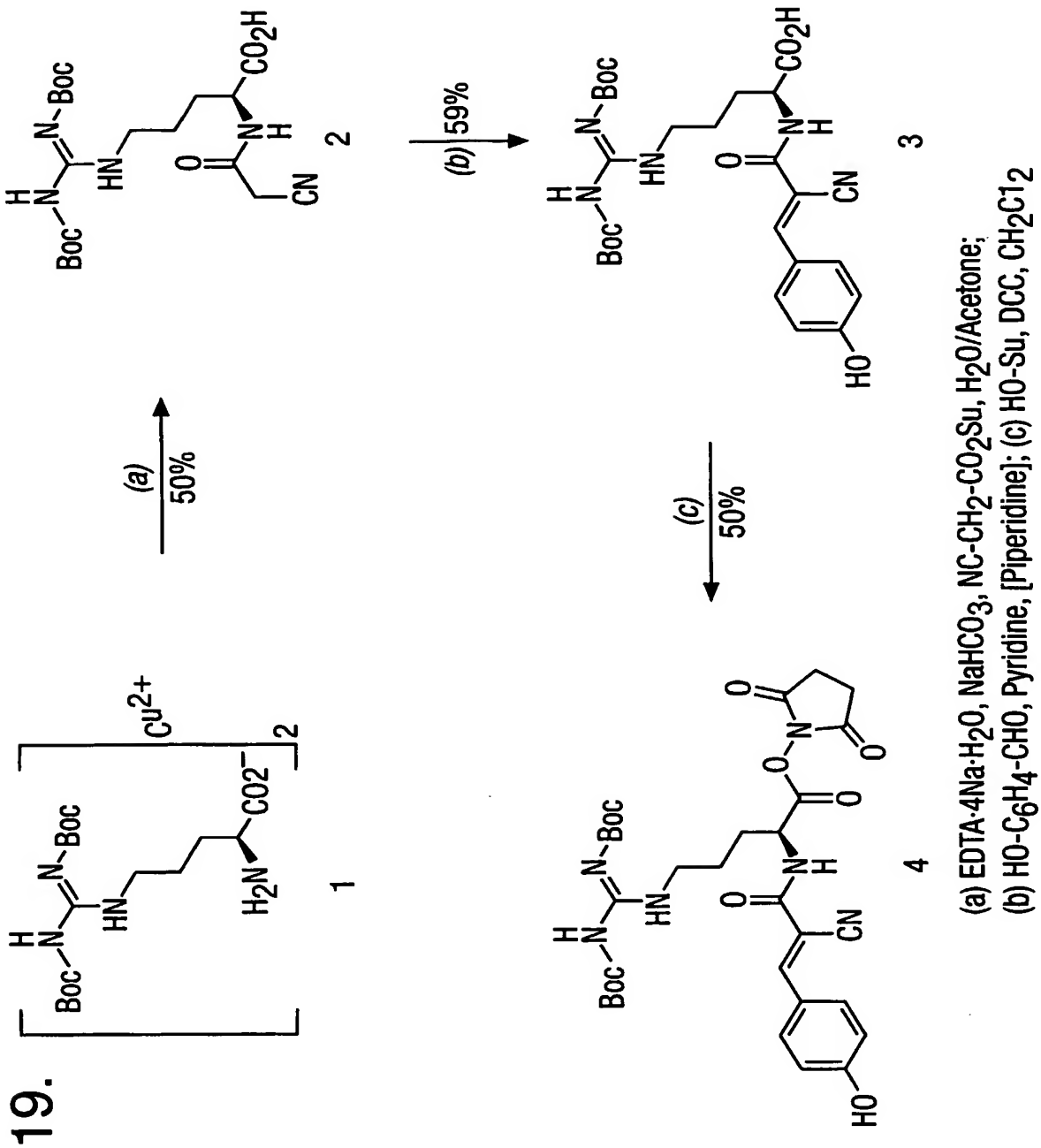
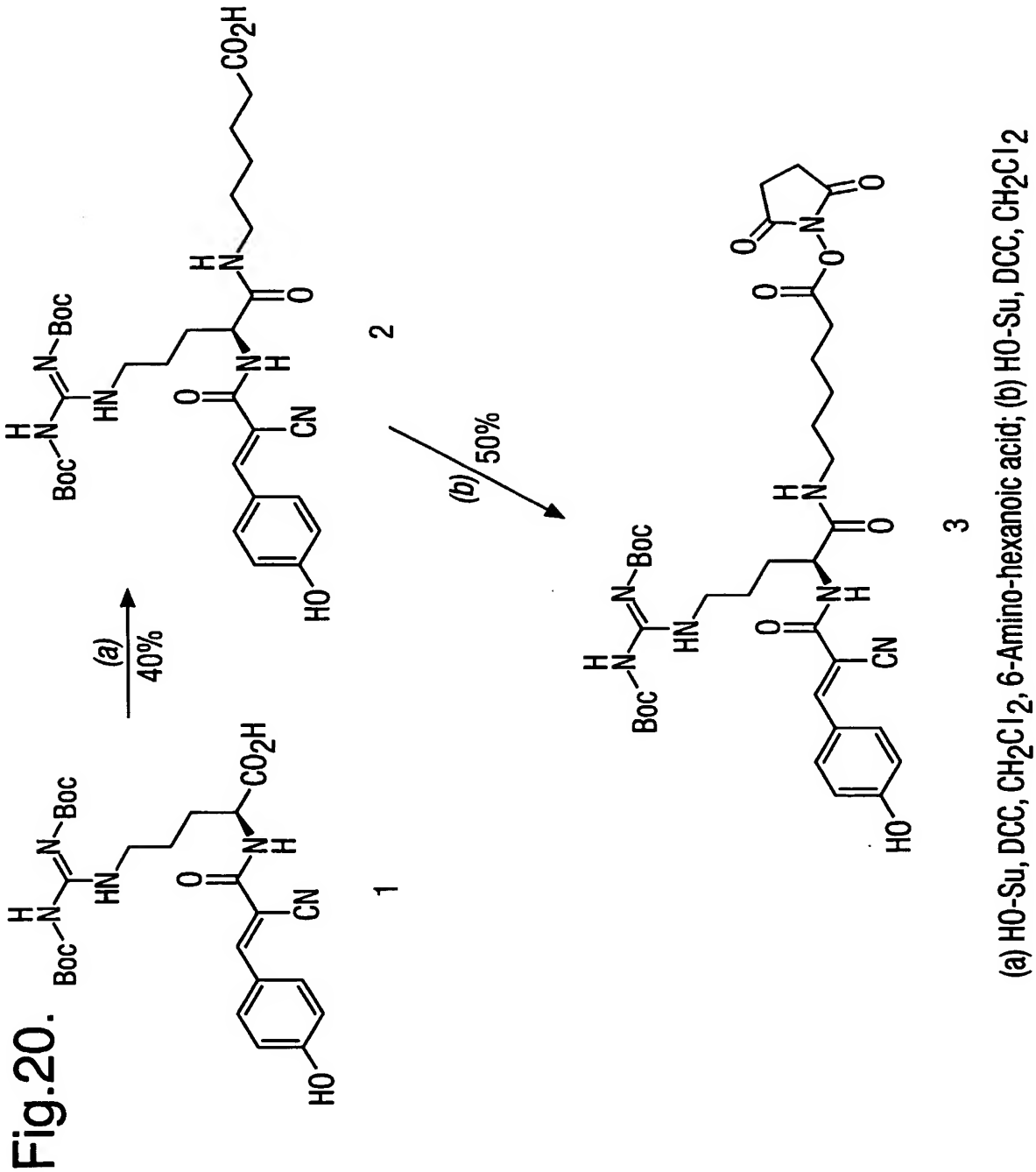

(a) EDTA·4Na·H2O, NaHCO3, NC-CH2-CO2Su, H2O/Acetone;
(b) HO-C6H4-CHO, Pyridine, [Piperidine]; (c) HO-Su, DCC, CH2C12
(a) 50%
(b) 59%
(c) 50%
Cu2+
Boc
HN
CO2H
CO2-
H2N
CN
HO
1
2
3
4

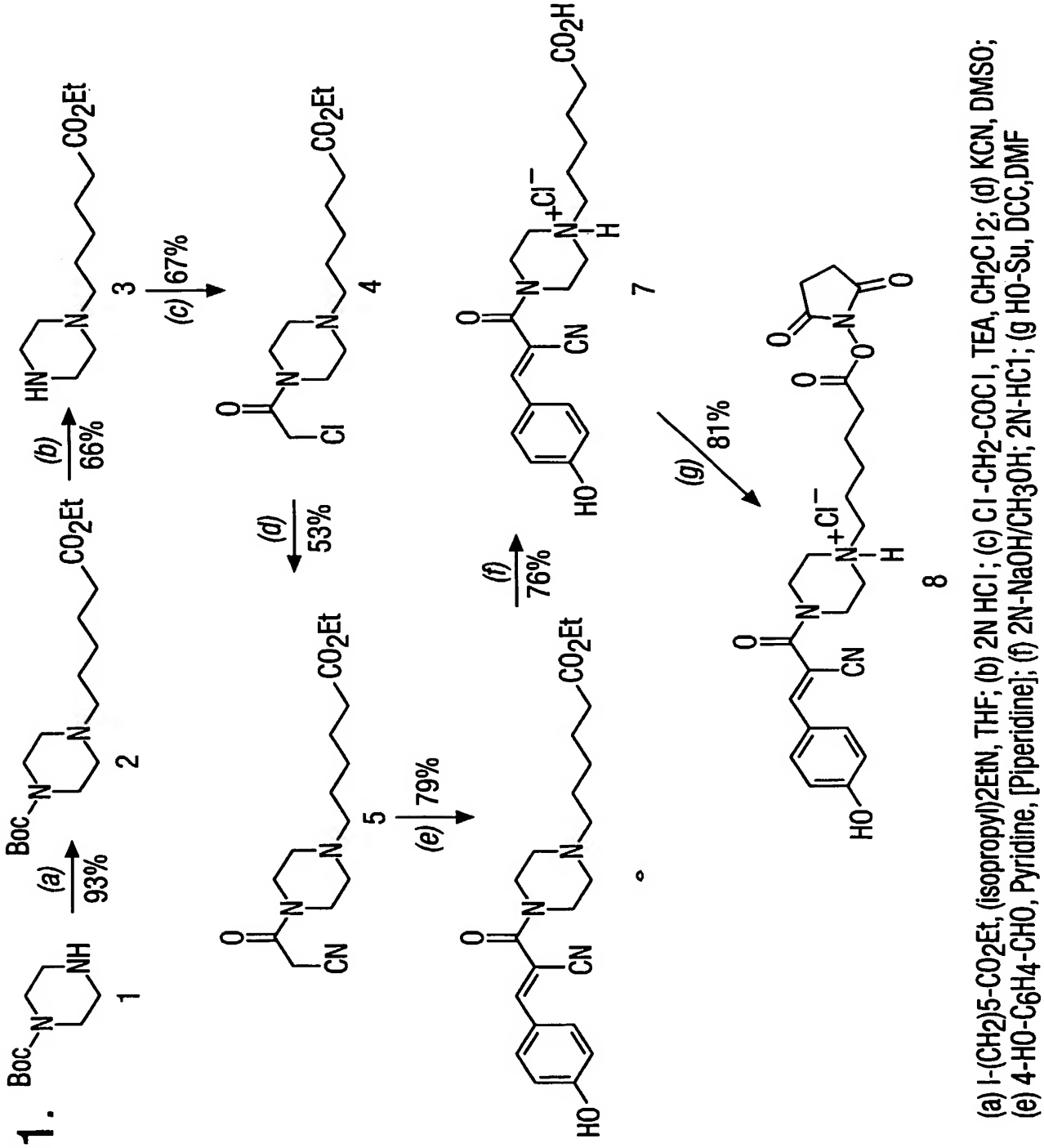
Fig.19.

Fig.20.



(a) HO-Su, DCC, CH_2Cl_2, 6-Amino-hexanoic acid; (b) HO-Su, DCC, CH_2Cl_2

Fig.21.



(a) I-(CH2)5-CO2Et, (isopropyl)2EtN, THF; (b) 2N HCl; (c) Cl-CH2-COCl, TEA, CH2Cl2; (d) KCN, DMSO;
(e) 4-HO-C6H4-CHO, Pyridine, [Piperidine]; (f) 2N-NaOH/CH3OH; 2N-HCl; (g HO-Su, DCC,DMF